PROSPECTIVE AND RETROSPECTIVE TESTING OF HIGH PREVALENCE HIV-1 SERUM AND BLOOD USING RAPID AND CONVENTIONAL TECHNOLOGY

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1. SUMMARY

We have conducted studies with existing rapid HIV-1 serology technology for applicability in military operations. Studies on fresh and frozen serum and differing HIV sub-types have been conducted in both the research and field environments. Testing has been compared to reference technology for calculation of operating characteristics. Conclusion: Rapid HIV-1 testing technology is an evolving field subject to market demands. Several tests exist that support warfighter use in the field. However, these tests should still be utilized in the context of the medical risk decision making process.

2. BACKGROUND

The US military conducts high-risk training and contingency operations worldwide. Providing medical assistance in resource limited environments endemic for serious infectious diseases poses challenges for health care workers. While the military may deploy with Level 3 medical assets during large operations, numerous lesser-supported deployments demonstrate the need for rapid point of care diagnostics. The potential for occupational exposure to blood and body fluids is high during combat and humanitarian operations. Unscreened blood products have been transfused into U.S. government personnel following exhaustion of pre-screened blood units during recent operations (Unpublished data, Division of Retrovirology, WRAIR). These exposures place soldiers at increased risk for disease transmission, to include HIV-1. HIV-1 prevalence in some areas of US operations is estimated as high as 30% (UNAIDS, 2003). Evaluating HIV-1 transmission risk includes the time critical determination of source patient seroreactivity. Performance of traditional HIV-1 serology by EIA and Western blot can take several hours to days or may be non-existent in resource-limited locations. Reflexive initiation of post-exposure prophylaxis (PEP) both consumes potentially small available stocks of antiretroviral drugs and subjects exposed workers to drug toxicity in situations were PEP is later determined by HIV-1 testing to not be indicated. This problem requires a portable, simple, and robust HIV-1 diagnostic device. Rapid HIV-1 testing can assist PEP decision making (van den Berk, et al. 2003) in these time-critical and supply constrained situations (Giles, et al. 1999; Henderson, 1999; MMWR, 1995).

3. MEDICAL APPLICATION

There are gaps present with HIV-1laboratory tests. HIV-1 reference serology is limited to EIA and Western Blot under non-portable conditions. There are three available rapid HIV-1 serology tests approved by the US Food and Drug Administration (FDA): Reveal (MedMira, Inc., Halifax, Nova Scotia), OraQuick HIV-1 (OraSure Technologies, Inc. Bethlehem, PA), and UniGold HIV-1 (Trinity Biotech, Wicklow, Ireland). Several other non FDA approved HIV-1 rapid tests are available overseas. While these tests are not confirmatory, they are simple to perform and claim high sensitivity and specificity. The operating characteristic for these tests must be optimal to ensure HIV-1 reactivity, or lack thereof, is determined. False positives must be reduced maximally to eliminate unnecessary prophylactic

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drug therapy and ensure limited medications are used to maximally reduce infectious risk.

Dendritic cells are infected within 24 hours in macaques mucosally exposed to SIV with migration to regional lymph nodes in the subsequent 24 to 48 hours (Spira, et al. 1996). PEP failed in this model when initiated beyond 48 hours (Tsai, et al. 1996). HIV-1 screening in voluntary counseling and testing settings requires diagnostic algorithms chosen on HIV-1 prevalence, serologic confounding factors, and cost constraints. Post-exposure testing must also return accurate test results to providers in time to guide rational PEP decisions.

4. MATERIALS AND METHODS

Prospective investigation was conducted utilizing fresh venous blood specimens from 486 study subjects during field testing in Kericho, Kenya. Retrospective testing was conducted with cryopreserved specimens randomly selected from among 14,000 HIV-1 EIA nonreactive and 1500 HIV-1 EIA reactive sera. This sera was collected from a HIV-1 seroprevalence study conducted in the Rakai District of Uganda (Wawer, et al. 1994) with a HIV-1 prevalence of 16.9% (Nalugoda, et al. 2004). Confirmatory testing had not been performed on any of these specimens. These samples were shipped frozen to the Division of Retrovirology, Walter Reed Army Institute of Research (WRAIR) in Rockville, MD where the serum was stored at -70°C. Rapid HIV-1 serologic testing was conducted using the following tests: UniGold HIV-1, OraQuick HIV-1, Reveal, Hemastrip (Saliva Diagnostic, Brooklyn, NY) and Determine (Abbott Laboratories, Inc., Abbott Park, IL). Results were compared to reference testing utilizing enzyme immunoassay (EIA) (Genetic Systems rLAV; Bio-Rad Laboratories, Redmond, Wash.). Repeatedly reactive specimens were tested by Western blotting (Cambridge Biotech HIV-1 Western blot; Calypte Biomedical Corp., Rockville, Md.). Viral load (VL) testing was performed with the Amplicor HIV-1 Monitor test version 1.5 (Roche Molecular Systems, Inc., Branchburg, N.J.) in standard mode.

This study was approved by the human use review boards of the WRAIR, the U.S. Army Medical Research and Material Command, the Uganda Virus Research Institute and the AIDS Research Subcommittee of the Uganda Council for Science and Technology, Study Number (M-1356).

Operating characteristics, predictive values, statistical simulation of the various permutations of rapid tests and the operating characteristics of the final testing algorithm of the retrospective study were calculated using STATA 7 (Stata Corporation, College Station, TX).

5. RESULTS

5.1 Prospective testing

All 62 HIV-1 specimens sero-positive by reference serology were reactive (true positive) by the OraQuick HIV-1, Determine HIV-1/2, and UniGold Recombigen HIV rapid tests. Sixty-one of these specimens were reactive by the Reveal HIV-1 test and the remaining such sample was not tested by oversight. There were no specimens positive by HIV-1 reference serology found to be non-reactive (false negative) by any of the four rapid tests. Results from 8 rapid test evaluations were reactive from 7 specimens non-reactive by reference serology (false positive). False reactive results were noted with OraQuick HIV-1 (3 specimens), Determine HIV-1/2 (4 specimens), and Reveal HIV-1 (1 specimen) testing. A single specimen was reactive with both the OraQuick HIV-1 and Reveal HIV-1 test. No false reactive results were observed with the UniGold HIV-1 test. Serum from all seven EIA non-reactive rapid test reactive specimens were subjected to testing with the Genetic Systems HIV-1 Western blot. A faint p18 band was observed with one specimen while the remaining six specimens were non-reactive. Quantitative HIV-1 RNA testing was below the level of detection (<400 copies/ml) on all seven specimens. See Table 1 for results.

5.2 Retrospective testing

Specimens for HIV-1 testing were subjected to both reference and rapid HIV-1 testing in two phases. The first phase included 1000 samples randomly selected from the pool of 15,500 samples. The results of the reference testing were compared to that of the rapid tests and descriptive epidemiological characteristics of the tests were calculated (Table 2). Hemastrip was insensitive (92.5%, 95% CI 90.8, 94.1) compared to the 100% sensitivity observed for the other three rapid tests.

OraQuick demonstrated the best specificity (97.6%,95%CI 96.6, 98.5), with both Determine and InstantScreen demonstrating specificities of <92%.

TABLE 1: PERFORMANCE OF RAPID HIV-1 PROSPECTIVE TESTING

	Test HIV-1 Positive		Test HIV-1 Negative				HIV-1 Prevalence = 0.128	
Test	No. of True* Positives (a)	No. of False* Positives (b)	No. of True* Negatives (d)	No. of False* Negatives (c)	Sensitivity (= a / a+c) (95% CI)	Specificity (= d / b+d) (95% CI)	Positive Predictive Value (= a / a+b) (95% CI)	Negative Predictive Value (= d / c+d) (95% CI)
OraQuick	62	3	421	0	100 (92.7 – 100)	99.3 (97.8 - 99.8)	95.4 (86.2 - 98.8)	100 (98.9 - 100)
Determine	62	4	420	0	100 (92.7 – 100)	99.1 (97.4 - 99.7)	93.9 (84.4 - 98.0)	100 (98.9 - 100)
UniGold	62	0	424	0	100 (92.7 – 100)	100 (98.9 - 100)	100 (92.7 – 100)	100 (98.9 - 100)
Reveal	61	1	423	0	100 (92.6 – 100)	99.8 (98.4 - 99.9)	98.4 (90.2 - 99.9)	100 (98.9 - 100)

* True and False HIV-1 seropositive status determined by Western Blot.

TABLE 2: PERFORMANCE OF RAPID HIV-1 RETROSPECTIVE TESTING

	Test HIV-1 Positive		Test HIV-1 Negative				HIV-1 Prevalence = 0.093	
Test	No. of True* Positive (a)	No. of False* Positive (b)	No. of True* Negatives (d)	No. of False* Negatives (c)	Sensitivity (= a / a+c) (95% CI)	Specificity (= d / b+d) (95% CI)	Positive Predictive Value (= a / a+b) (95% CI)	Negative Predictive Value (= d / c+d) (95% CI)
OraQuick	885	0	22	93	100 (100, 100)	97.6 (96.6, 98.5)	80.9 (78.4, 83.3)	100 (100, 100)
Hemastrip	857	7	50	86	92.5 (90.8, 94.1)	94.5 (93.1, 95.9)	63.2 (60.3, 66.2)	99.2 (98.6, 99.8)
Instant Screen	833	0	74	93	100 (100, 100)	91.8 (90.1, 93.5)	55.7 (52.6, 58.8)	100 (100, 100)
Determine	832	0	75	93	100 (100, 100)	91.7 (90.0, 93.4)	55.4 (52.3, 58.4)	100 (100, 100)

* True and False HIV-1 seropositive status determined by Western Blot.

TABLE 1 & 2: The number of true and false positive and negative results for each of four HIV-1 rapid tests are given above. These values were used to compute sensitivity, specificity, and positive and negative predictive values (expressed as percentage) using the indicated observed prevalences for HIV-1 infection using HIV-1 EIA and Western blotting as the reference testing modality. Computed values are given along with 95% confidence intervals.

Hypothetical evaluation of three-test serial and parallel testing algorithms were performed. The serial design (Figure 1A) employs a single screening test. If that test was non-reactive, the sample was considered HIV-1 negative. If the initial test was reactive, a second test was conducted. An HIV-1 positive final result was assigned in the case of concordance between the two tests. A third test was conducted in cases of discordant results. The result of the third test determined HIV-1 status. The parallel design (Figure 1B) utilized two tests performed simultaneously. Concordant reactive or non-reactive results determined HIV-1 positive or negative results, respectively. The third test was utilized for discordant test pairs. The result of the third test determined HIV-1 status. All hypothetical algorithms inclusive of OraQuick, Instant Screen and Determine performed equally well. However, the overall choice of a design was made based on time-to-results and concern for false negatives results(van den Berk, et al. 2003). The best performing algorithms displayed operating characteristics as follows: sensitivity =100%, specificity=99.6 (95%CI 99.5,100), positive predictive value (PPV) =97.9 (95%CI 97.0,98.8) and negative predictive value (NPV) =100%, prevalence =9.3%). The decision to utilize the OraQuick-Determine; Instant Screen parallel algorithm was based on

ease of use and reduced time-to-results (22 versus 35 minutes). (Table 3). A parallel algorithm carries the theoretic advantage of increased screening sensitivity by diversification by initial test biochemistry. Utilizing the higher specificity test earlier in the hypothetical algorithm did not affect the outcome.

3,500 samples were randomly selected from within the remaining pool of 14,500 specimens, and subjected to the OraQuick-Determine; Instant Screen parallel algorithm and compared to the reference serology. 474 specimens were concordantly HIV-1 positive, 2993 specimens concordantly HIV-1 negative, and 33 specimens were false positive by the rapid test algorithm (Table 3). There were no false negative results. This describes a sensitivity of 100% (95% CI= 100,100%) and a specificity of 98.9% (95%CI= 98.6, 99.3%). The PPV and NPV, based on a prevalence of 13.5% were 93.5% (95% CI= 92.7, 94.3%) and 100% (95%= 100, 100%) respectively.

A fraction of the seropositive and seronegative samples from the second phase of the study were subjected to HIV-1 viral load testing to qualitatively correlate serology to viremia and control for possible early seronegative HIV-1 infection (Table 4). All 92 specimens concordantly seronegative by both reference and rapid test algorithms were had no detective HIV-1 RNA. Thirty-three specimens were negative by reference serology but positive by rapid testing. All 25 specimens available for testing from this had no detectable HIV-1 RNA. Ninety-one of 96 specimens concordantly positive by both traditional and rapid testing were positive by HIV-1 RNA testing, but five such samples had no detectable HIV-1 RNA consistent with robust host viral control(Birx, et al, 2000, Mellors, et al, 1996) or sample degradation. This raises questions as to utility of the HIV-1 RNA testing used in these experiments for supplemental HIV-1 testing.

FIGURE 1A

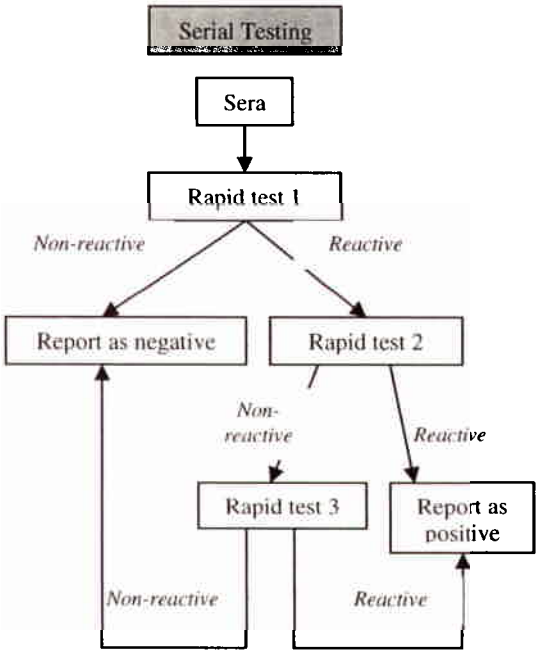

FIGURE 1B

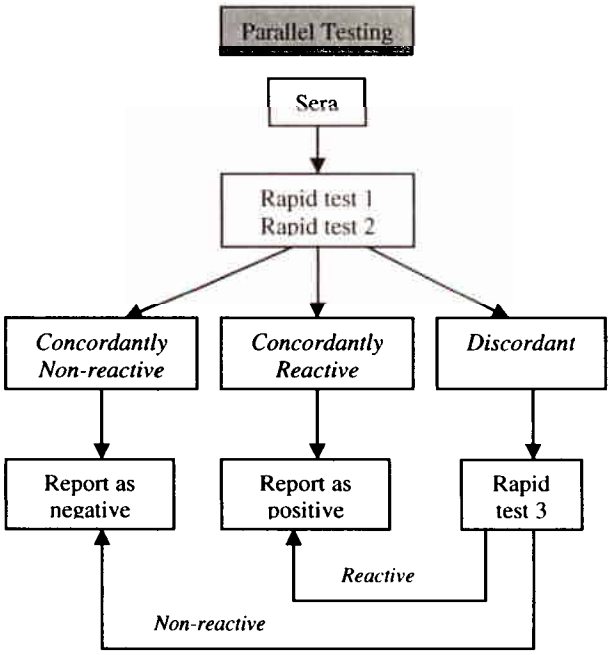

TABLE 3: CALCULATED OPERATING CHARACTERISTICS BASED HYPOTHETICAL ALGORITHMS OF HIGHEST PERFORMING MODELS

Design	Algorithm	Sensitivity (95% CI)	Specificity (95% CI)	Time to Results
Parallel	Ora- Det; InScr	100% (100,100)	99.6% (99.0, 99.9)	22 minutes
Parallel	Det- InScr; Ora	100% (100,100)	99.6% (99.0, 99.9)	35 minutes
Serial	Det- InScr; Ora	100% (100,100)	99.6% (99.2, 100)	37 minutes
Serial	InScr- Ora; Determine	100% (100,100)	99.6% (99.0, 99.9)	37 minutes
Serial	Ora-Det; InScr	100% (100,100)	99.6% (99.0, 99.9)	37 minutes

Table 3: Determined by operating characteristics of 1000 samples (see Table 2) and calculated by statistical modeling (STATA). Time-to-results were calculated by actual performance of each algorithm. CI, confidence interval; Ora, Oraquick; InScr, Instant Screen; Det, Determine.

TABLE 4: DESCRIPTIVE CHARACTERISTICS OF THE PARALLEL TESTING ALGORITHM ORAQUICK-DETERMINE; INSTANT SCREEN.

		Reference Standard		Total
		Reactive	Non-Reactive	
Rapid algorithm	Reactive	474	33	507
	Non-react	0	2993	2993
Total		474	3026	3500

Table 4: 3500 samples were randomly selected from the remaining pool of 14,500 samples (after 1000 had been selected for the first phase of the study). These samples were subjected to the OraQuick-Determine; InstantScreen parallel rapid test algorithm and compared to reference serology: sensitivity=100% (95% CI= 100,100%), specificity=98.9% (95%CI= 98.6, 99.3%). Predictive values based on observed prevalence of 13.5% (as measured by traditional serology). PPV=93.5% (95% CI= 92.7, 94.3%); NPV=100% (95%= 100, 100%).

TABLE 4: VIRAL LOAD DETERMINATION OF SELECTED SAMPLES

	Concordant Reactive	Concordant Nonreactive	Discordant*	False Negatives
Total (from Table 3)	474	2293	33	0
Total Tested for VL	96	92	25	N/A
Detectable VL	91	0	0	N/A
Below Detection	5	92	25	N/A

Table 4: Viral load determination (Amplicor HIV-1 Monitor test version 1.5) of selected samples. 96 samples were randomly selected from 474 concordantly seropositive (reference and rapid algorithm) testing, of which 91 had a detectable viral load. 92 samples were randomly selected from 2293 concordantly seronegative (reference and rapid algorithm) from the rapid algorithm evaluation phase (3500 samples), of which all 92 samples were determined to be below detection for viral load. Testing produced 33 reference testing seronegative, rapid algorithm seropositive samples, of which 25 were subjected to viral load determination (8 of the 33 samples had insufficient volume available for testing), of which all 25 were determined to be below the level of detection. * (Reference testing Non reactive, rapid algorithm reactive)

6. CONCLUSIONS

Parallel testing is not recommended by the WHO for routine HIV-1 screening (HIV Rapid Tests, 2004). However, post-exposure HIV-1 testing addresses different testing requirements. An ideal post-exposure testing algorithm would include differing antigen sources and different test principles (Stickle, et al, 2002; Urassa, et al, 1999) to eliminate the possibility of a false negative (van den Berk, et al. 2003), and to provide accurate serologic results within the shortest time (HIV Rapid Tests, 2004; PHS Guidelines, 2001). This approach, in combination with risk factor

analysis, could reduce the risk of unnecessary pharmacologic toxicities (PHS Guidelines, 2001) in post-exposure scenarios. This is especially relevant in resource limited environments where available antiretroviral drugs are scarce.

The nature of military operations with potential exposure to body fluids requires field assessment of HIV-1 exposure risk. The choice of HIV-1 diagnostic algorithm to use will be influenced by HIV-1 prevalence, population-specific serologic confounding factors, governmental regulatory issues, and cost. Rapid HIV testing is optimal when diagnostic timing is acute (occupational exposure) or when compounded by resource limited environments (limited supply of antiretrovirals). The great promise of rapid HIV-1 antibody screening tests are their ability to provide highly sensitive, specific and rapid (<30 minute) sero-diagnosis in settings where resources and support are limited. We hope to improve the laboratory support for Level I and II providers and reduce the risk of an unnecessary pharmacologic exposure or risks for transfusion transmission of infectious disease.

REFERENCES

HIV Rapid Tests: Guidelines for Use in HIV Testing And Counseling Services In Resource-Constrained Settings. 2004. http://www.who.int/hiv/pub/vct/en/rapidhivtestsen.pdf.

Update: HIV counseling and testing using rapid tests--United States, 1995. MMWR 47:211-215.

Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations for Postexposure Prophylaxis. 2001. MMWR **50**:1-52.

Giles RE, P. K., and Parry JV. 1999. Simple/rapid test devices for anti-HIV screening: do they come up to the mark? J. Med. Virol. 59:104-109.

Henderson, D. K. 1999. Postexposure chemoprophylaxis for occupational exposures to the human immunodeficiency virus. JAMA 281:931-6.

Nalugoda, F., R. H. Gray, D. Serwadda, N. K. Sewankambo, F. Wabwire-Mangen, N. Kiwanuka, T. Lutalo, G. Kigozi, C. Li, F. Makumbi, M. Kiddugavu, L. Paxton, S. Zawedde, and M. Wawer. 2004. Burden of infection among heads and non-head of rural households in Rakai, Uganda. AIDS Care 16:107-15.

Spira, A. I., P. A. Marx, B. K. Patterson, J. Mahoney, R. A. Koup, S. M. Wolinsky, and D. D. Ho. 1996. Cellular targets of infection and route of viral dissemination after an intravaginal inoculation of simian immunodeficiency virus into rhesus macaques. J Exp Med 183:215-25.

Stickle, D. F., S. J. Pirruccello, S. Swindells, and S. H. Hinrichs. 2002. Discrepant results of 2 screening tests for anti-HIV antibody. Clin Infect Dis 35:773-4.

Tsai CC, E. P., Follis KE, Beck TW, Benveniste RE, Bischofberger N, Lifson JD, Morton WR. 1998. Effectiveness of postinoculation (R)-9-(2-phosphonylmethoxypropyl) adenine treatment for prevention of persistent simian immunodeficiency virus SIVmne infection depends critically on timing of initiation and duration of treatment. J Virol 72:4265-4273.

Urassa, W., K. Godoy, J. Killewo, G. Kwesigabo, A. Mbakileki, F. Mhalu, and G. Biberfeld. 1999. The accuracy of an alternative confirmatory strategy for detection of antibodies to HIV-1: experience from a regional laboratory in Kagera, Tanzania. J Clin Virol 14:25-9.

UNAIDS Office of AIDS, Security and Humanitarian Response, 2003: Joint United Nations Program on HIV/AIDS, On the Front Line. UNAIDS/03.44E.

van den Berk, G. E. and P. H. Frissen, R. M. Regez, and P. J. Rietra. 2003. Evaluation of the rapid immunoassay determine HIV 1/2 for detection of antibodies to human immunodeficiency virus types 1 and 2. J Clin Microbiol 41:3868-9.

Wawer, M. J., N. K. Sewankambo, S. Berkley, D. Serwadda, S. D. Musgrave, R. H. Gray, M. Musagara, R. Y. Stallings, and J. K. Konde-Lule. 1994. Incidence of HIV-1 infection in a rural region of Uganda. BMJ 308:171-3 .